PUBLIC HEALTH REPORTS

VOL. 33 JUNE 21, 1918 No. 25

Influenza a Probable Cause of Fever of Undetermined Nature in Southern States.

Fevers of an undetermined nature were reported during April and May at various points from Norfolk to Louisiana. An examination of the records and reports of the physicians who have treated these cases leads to the belief that these fevers were mainly influenza of mild type.

It is possible, however, that all cases reported were not of the same disease, and in one locality in Louisiana dengue may have occurred.

North Carolina Enforcing Law Requiring Morbidity Reports.

A determined effort is being made by the State Board of Health of North Carolina to secure the reporting of cases of communicable disease by physicians throughout the State and the prompt transmission of the reports to the State Board of Health.

During the week ended June 8, 1918, two physicians were prosecuted and fined for failure to report cases of notifiable diseases as required by the State law. A county quarantine officer was also prosecuted for failure to perform the duties of his office. He pleaded guilty, and the case was dismissed upon his promise to comply with the law in the future.

Some Qualitative and Quantitative Tests for Arsphenamine (3, 3′-Diamino-4, 4′-Dioxy-Arsenobenzene Dihydrochloride) and Neo-Arsphenamine (Sodium-3, 3′-Diamino-4, 4′-Dihydroxy-Arsenobenzene-Methanal-Sulphoxalate).

By C. N. MYERS, Organic Chemist, and A. G. DUMEZ, Technical Assistant, Hygienic Laboratory, United States Public Health Service.

Previous to the year 1914, all of the arsphenamine (salvarsan) and neo-arsphenamine (neosalvarsan) on the market was manufactured by a single German firm under the supervision of Paul Ehrlich, one of the patentees. Naturally the products were fairly uniform in their composition and properties.

As a result of the present war in Europe, the protection afforded these products in the allied countries, through licenses or patents, has been temporarily withdrawn, and they are now being manufactured in England, France, Japan, Canada, and the United States.

Examinations made by the authors, as well as evidence presented by clinicians (Martin and others, 1916), have revealed the fact that the products of different manufacturers appearing on the market in this country are not all uniform with respect to either their chemical or their physiological properties. Even the last of the German supplies received are stated to be more toxic than the products obtained before the beginning of hostilities in Europe (Ormsby and Mitchell, 1916).

Tentative standards for these preparations (arsphenamine and neo-arsphenamine) have been adopted by the Federal Trade Commission on the recommendation of the United States Public Health Service, but these do not appear to meet all exigencies. It is for this reason and for the purpose of better defining the properties of good preparations that the following qualitative and quantitative tests have been worked out and compiled.

Arsphenamine—Physical Properties.

Appearance: Arsphenamine is a pale yellow, microcrystalline, hygroscopic powder very unstable in the air. When properly dried, it is free from lumps.

Odor: The pure product is odorless.[1]

Taste: It has a sour astringent taste.

Solubility: Arsphenamine is soluble in water, 1 to 5 parts, methyl alcohol, 1 to 3 parts, and ethyl alcohol, 1 to 12 parts (Wilcox and Webster, 1916). It is readily soluble in ethylene glycol and glycerin, but only slightly soluble in glacial acetic acid, acetone, ether and concentrated hydrochloric acid (Ehrlich and Bertheim, 1912).

The aqueous solution is greenish-yellow[2] in color and reacts strongly acid to litmus.

Moisture content: When dried to constant weight in an atmosphere of dry hydrogen at 105° C., arsphenamine should lose not more than 7.6 per cent of its weight, which corresponds to the loss of 2 molecules of water of crystallization (Gaebel, 1911).

Arsphenamine—Chemical Properties.

Behavior toward acids: Dilute mineral acids, with the exception of dilute sulphuric acid, have no noticeable effect on aqueous solu-

[1] Commercial samples frequently have the odor of ether due to the incomplete removal of this solvent which is used in precipitating and washing the product.

[2] The brownish-yellow or brown color, sometimes observed in solutions prepared from commercial samples, is thought to be an indication of the presence of oxidation products or other impurities.

tions of arsphenamine[1] (distinction from *neo-arsphenamine*, which yields a precipitate with all dilute mineral acids).

The addition of dilute sulphuric acid, however, produces a yellowish-white precipitate.[2]

The addition of any of the concentrated mineral acids, with the exception of phosphoric, to an aqueous solution of arsphenamine causes the formation of a precipitate (distinction from *neo-arsphenamine*, which is precipitated by phosphoric acid).

In the case of concentrated nitric acid, the precipitate dissolves on the addition of an excess of acid yielding a red solution.

Acetic acid (36 per cent) produces no noticeable effect when added to an aqueous solution of arsphenamine (distinction from *neo-arsphenamine*, which yields an orange-yellow precipitate on heating the liquid).

Carbon dioxide immediately precipitates arsphenamine from aqueous solutions.

Behavior toward alkalies: The addition of sodium hydroxide test solution to an aqueous solution of arsphenamine produces a precipitate which dissolves in an excess of the reagent.[3]

Solutions of barium and calcium hydroxides also yield precipitates.

The alkali carbonates produce precipitates which are not soluble in an excess of the reagent.

Behavior toward oxidizing agents: The addition of chlorine or bromine water, ferric chloride, or chromic acid to an aqueous solution of arsphenamine causes the liquid to become red or brownish red in color.

Behavior toward general alkaloidal reagents: An aqueous solution of arsphenamine slowly reduces gold and platinic chloride test solutions in the cold, yielding characteristic precipitates. Reduction is hastened by heating.

Mercuric chloride test solution produces a light-yellow colored precipitate which becomes white on heating.

Mayer's reagent gives a heavy, orange-yellow precipitate.

Picric acid test solution produces a copious yellow precipitate (distinction from *neo-arsphenamine*, aqueous solutions of which become only slightly turbid on the addition of picric acid test solution).

---

[1] For carrying out the above tests, or those which follow, a 1 in 1,000 aqueous solution of the product was used, unless otherwise mentioned.

All of the test solutions employed were made according to the U. S. P. IX, unless differently stated.

[2] Precipitation also occurs on the addition of sulphates.

[3] Precipitation first begins when 1 mol of sodium hydroxide has been added for each mol of arsphenamine in solution. If the addition of sodium hydroxide is continued until precipitation is complete, a further addition of alkali will cause the precipitate to go into solution as the phenolate (Ehrlich and Bertheim, 1912).

Phosphotungstic acid test solution [1] produces a dirty gray colored precipitate, insoluble in an excess of the reagent, but which dissolves upon the addition of sodium carbonate, or ammonia water, yielding a deep blue colored solution.

Phosphomolybdic acid test solution gives a similar color reaction if the liquid is made acid with hydrochloric acid after the addition of the alkali (Gaebel, 1911b).

Behavior toward other reagents: The addition of a freshly prepared solution of ferric chloride and potassium ferricyanide to an aqueous solution of arsphenamine immediately produces a copious precipitate of Prussian blue.

Nessler's reagent is instantly reduced.

The addition of silver nitrate test solution first causes a yellow color to appear, then the formation of a gelatinous precipitate which changes to a black powder on heating. The black precipitate is soluble in dilute nitric acid.

Millon's reagent gives a copious yellow precipitate.

If a drop of copper sulphate solution (4 in 100) be added to 5 cubic centimeters of an aqueous solution of salvarsan (1 in 1,000), to which has been added 0.5 cubic centimeter of hydrogen dioxide solution and 0.5 cubic centimeter of ammonia water, an intense bluish-green color will develop. If the blue solution is poured into alcohol (90 per cent), a blue precipitate, which can be separated by centrifugation, will be obtained (Denigès and Labat, 1911).

To 2 or 3 cubic centimeters of an aqueous solution of arsphenamine (1 in 1,000) add 3 or 4 drops of dilute hydrochloric acid (an amount sufficient to cause the disappearance of most of the yellow color), cool the solution by holding the test tube in ice water and add 3 or 4 drops of a solution of sodium nitrite (5 in 1,000). This results in the formation of a diazo compound having a greenish-yellow fluorescence (distinction from *neo-arsphenamine*, which forms a brown solution).

If a small portion of the solution containing the diazo compound be added drop by drop to an alcoholic solution of α-naphthylamine hydrochloride, a beautiful violet color will develop (Gaebel, 1911b).

With an alcoholic solution of β-naphthylamine hydrochloride, a light-brown color develops (distinction from *atoxyl*, which yields a red-colored solution, Wilcox and Webster, 1916).

If some of the diazotized solution be added to a freshly prepared solution of resorcinol (1 part in 20 parts of a 10 per cent sodium hydroxide solution), a deep red color will develop (Abelin, 1911).

The direct addition of Ehrlich and Pauly's (1904) diazo reagent to an aqueous solution of arsphenamine produces a brownish-red color.

[1] The phosphotungstic acid solution used in the above test was prepared according to the method of Folin and Denis (1912).

Tests for arsenic: A positive test for arsenic is obtained by applying the Reinsch test.

The Marsh test gives positive results if the arsphenamine is first decomposed by oxidation with nitric and sulphuric acids and the resulting solution reduced by the addition of potassium meta-bisulphite (Wilcox and Webster, 1916).

Under the foregoing conditions, the Gutzeit's test also gives positive results.

The biological test with *Penicillium brevicaule*, carried out according to the method of Abel and Buttenberg, gives the characteristic garlic odor (Gaebel, 1911b).

Tests for impurities: An aqueous solution of arsphenamine yields no precipitate with hydrogen sulphide, even after the addition of hydrochloric acid and warming (absence of *inorganic arsenic compounds*).

If 4 cubic centimeters of sodium acetate test solution are added to 5 cubic centimeters of an aqueous solution of arsphenamine (1 in 10), the mixture heated for a few minutes and the precipitate removed by filtration, the filtrate should not yield a precipitate within 12 hours on being made alkaline with 3 cubic centimeters of ammonia water and the addition of magnesia mixture (absence of *inorganic arsenic compounds*, Moeller and Thoms, 1914).

If about 0.1 gram of arsphenamine be placed in a test tube, a small quantity of zinc dust and some dilute hydrochloric acid added,[1] and the mouth of the tube covered with a piece of filter paper moistened with a 5 per cent solution of cadmium chloride, the paper should not be stained yellow within a few minutes (absence of *sulphur compounds*).[2]

Dissolve exactly 1.0 gram of arsphenamine in 10 cubic centimeters of methyl alcohol contained in a 100 cubic centimeter volumetric flask. Dilute the solution with 75 cubic centimeters of distilled water, add 1.5 grams of precipitated calcium carbonate, and shake to precipitate the salvarsan base. Dilute with distilled water to exactly 100 cubic centimeters and filter. To exactly 50 cubic centimeters of the filtrate add 75 cubic centimeters of water, 5 cubic centimeters of N/1 hydrochloric acid volumetric solution, and titrate with N/20 iodine volumetric solution. The amount of iodine volumetric solution consumed, expressed in cubic centimeters, represents the percentage of *amino-oxy-phenyl-arsenoxide* present in the material. The

[1] A drop of platinic chloride test solution may be added to start the reaction.

[2] Arsinsulphide and Arsinsesquisulphide have been suggested as possible impurities in arsphenamine (Schamberg, Kolmer, and Raizies, 1917).

Most of the commercial samples of arsphenamine examined in this laboratory gave a positive test for sulphur by the method described above.

amount of the oxide present in good products varies from 0.5 to 0.8 per cent [1] (Ehrlich and Bertheim, 1912).

Neo-Arsphenamine—Physical Properties.

Appearance: Neo-arsphenamine is an orange-yellow, microcrystalline powder which changes rapidly in the air, becoming dark brown in color.

Odor: The pure preparation is odorless.[2]

Taste: It has a taste somewhat resembling that of garlic.[3]

Solubility: Neo-arsphenamine is readily soluble in water or glycerin, but only slightly soluble in methyl alcohol, ethyl alcohol, acetone, and ether.

The aqueous solution, when freshly prepared, is yellow in color and reacts neutral toward litmus. The solution rapidly becomes brown on exposure to the air.

Neo-Arsphenamine—Chemical Properties.

Behavior toward acids: Dilute as well as concentrated mineral acids yield precipitates with an aqueous solution of neo-arsphenamine. Precipitation does not occur immediately, but is first noticeable after several minutes (distinction from *arsphenamine*, which is not precipitated by dilute mineral acids or concentrated phosphoric acid, but yields a precipitate immediately with concentrated hydrochloric, sulphuric, and nitric acids).

The addition of acetic acid (36 per cent) to an aqueous solution of neo-arsphenamine yields a yellow colored precipitate when the liquid is heated (distinction from *arsphenamine*, which is not precipitated).

Behavior toward alkalies: The addition of sodium hydroxide test solution to an aqueous solution of neo-arsphenamine produces no noticeable effect (distinction from *arsphenamine*, a solution of which yields a precipitate).

Solutions of barium and calcium hydroxides yield turbid solutions or faint precipitates.

Solutions of the alkali carbonates do not produce precipitates (distinction from *arsphenamine*).

Behavior toward oxidizing agents: Similar to the reactions with *arsphenamine*.

Behavior toward general alkaloidal reagents: Similar to the reactions with *arsphenamine*, except that the precipitate with picric acid test solution develops slowly and is relatively small in amount.

---

[1] The amount of oxide found in the commercial samples examined in this laboratory varied from 0.5 to 2.8 per cent.

[2] Commercial samples sometimes have an odor of garlic, due apparently to slight decomposition.

[3] Commercial samples frequently have a saline taste, probably due to the presence of sodium chloride which is said to be used as a diluent for products high in arsenic content.

Mayer's reagent does not yield a precipitate until the solution has been made acid with dilute hydrochloric acid (distinction from a solution of *arsphenamine,* which yields a precipitate on the direct addition of the reagent).

Behavior toward other reagents: The behavior of an aqueous solution of neo-arsphenamine toward a freshly prepared solution of ferric chloride and potassium ferricyanide, silver nitrate test, and Nessler's reagent is similar to that described under *arsphenamine.*

Millon's reagent yields a copious brown-colored precipitate.

If 5 cubic centimeters of dilute hydrochloric acid be added to 10 cubic centimeters of an aqueous solution of neo-arsphenamine (1 in 100) and the mixture heated, the irritating odor of sulphur dioxide will be developed (New and Nonofficial Remedies, 1917).

If about 0.1 gram of neo-arsphenamine be placed in a test tube, a small quantity of zinc dust and some dilute hydrochloric acid added and the mouth of the tube covered with a piece of filter paper moistened with a 5 per cent solution of cadmium chloride, the paper will be stained yellow within a few minutes (distinction from *arsphenamine*).

If 5 cubic centimeters of an aqueous solution of neo-arsphenamine be boiled with 1 cubic centimeter of dilute hydrochloric acid, a violet color will develop on the addition of a few drops of Schiff's reagent [1] (distinction from *arsphenamine,* Denigès and Labat, 1913).

The diazotized solution [2] of neo-arsphenamine gives color reactions with α-naphthylamine hydrochloride and resorcinol similar to those described under *arsphenamine.* With β-naphthylamine hydrochloride, a brownish-red color develops.

Tests for arsenic: The reactions are similar to those noted under *arsphenamine.*

Tests for impurities: An aqueous solution of neo-arsphenamine [3] yields no precipitate on passing in hydrogen sulphide gas (absence of *inorganic arsenic compounds*).

If 5 cubic centimeters of acetic acid (36 per cent) be added to 5 cubic centimeters of an aqueous solution of neo-arsphenamine, the mixture heated a few minutes and the precipitate removed by filtration, the filtrate should not yield a precipitate within 12 hours on the addition of an excess of ammonia water and some magnesia mixture (absence of *inorganic arsenic compounds*).

---

[1] By boiling with hydrochloric acid, the methylene group of the neo-arsphenamine is detached and oxidized to formic aldehyde.

[2] In diazotizing the solution, add the sodium nitrite solution first, then the hydrochloric acid in order to avoid precipitation.

[3] Hydrochloric acid should not be added, as acids produce a precipitate.

Arsphenamine and Neo-Arsphenamine—Quantitative Determination of Arsenic.

The methods for the quantitative determination of arsenic in organic compounds, described in the literature, are both numerous and varied in their manner of execution. Most of them, however, are more or less complicated and are, therefore, not suitable for use in routine work where the number of samples of material to be analyzed is large. They involve, for example, such processes as fusion (methods of La Coste and Michaelis, 1880; of Pringsheim, 1904; of Little, Cahen, and Morgan, 1909; and of St. Warunis, 1912); or distillation (methods of Schneider and Fyfe, 1906; of Jannasch and Seidel, 1910; and of Bohrisch and Kürschner, 1911); and the subsequent estimation of the arsenic by gravimetric or volumetric methods.

Among the simpler and more practical procedures, which have received special mention in connection with the estimation of the arsenic in arsphenamine or neo-arsphenamine, are the methods of Gaebel (1911c) and Denigès and Labat (1911), in which an aqueous solution of the material is titrated directly with iodine or potassium permanganate volumetric solution. In this class are, likewise, the methods of Norton and Koch (1905), Lehmann (1912), and Ewins (1916). In these methods the arsenic is, first, either oxidized or reduced by digesting the material with suitable reagents and then estimated by titration in one of the usual ways.

For the purpose of determining which one of these simpler methods is the most accurate, and can be depended upon to give the best results in the hands of different operators, a few preliminary analyses were carried out. The results obtained indicated that the methods of Gaebel, Ewins, and Lehmann offered the greatest possibilities for fulfilling these conditions.[1] A large number of samples of both arsphenamine and neo-arsphenamine were, therefore, subjected to analysis by these methods. For comparison, a number of gravimetric determinations were also made. Detailed descriptions of these methods, together with the data obtained in the analyses, follow:

Gaebel's titration method: Weigh out accurately about 0.2 gram of arsphenamine and dissolve it in 100 cubic centimeters of distilled water contained in an Erlenmeyer flask. Add 1 cubic centimeter of starch test solution and titrate with N/20 iodine volumetric solution to a permanent blue color.[2] One cubic centimeter of N/20 iodine volumetric solution is equivalent to 0.001875 gram of arsenic.

[1] The method of Denigès and Labat was eliminated from the field of possibilities, as the end point obtained in the titration is too indefinite to yield accurate results in the hands of different analysts.

The Ewins method was given preference over that of Norton and Koch, as it is essentially an improved modification of the latter

[2] As the greenish-yellow color of the arsphenamine solution becomes less and less pronounced and finally vanishes on the addition of iodine solution, the titration may also be carried out without the use of an indicator.

Ewins's method: Weigh out accurately 0.1 to 0.2 gram of the substance and transfer it to a long-necked Kjeldahl flask of 300 cubic centimeters capacity. Add 10 grams of potassium sulphate and 0.2 to 0.3 gram of starch (after a little experience the amount can be sufficiently accurately estimated and need not be weighed). Wash in any solid adhering to the neck of the flask with a little water. Cautiously add 20 cubic centimeters of concentrated sulphuric acid and heat the mixture on wire gauze over a Bunsen flame. As soon as the contents of the flask begin to froth, lower the flame somewhat until the frothing diminishes, which generally takes place within 10 to 15 minutes from the commencement of heating. Again turn on the flame and continue heating until the liquid becomes colorless or of a very pale yellow tint. Shake the flask once or twice during digestion, in order to wash down any material adhering to the walls. The time required for the complete oxidation of the material is usually about 4 hours.

After the liquid has cooled, transfer it quantitatively to an Erlenmeyer flask of 350 cubic centimeters capacity and make it just distinctly alkaline by the addition of sodium hydroxide solution (10 to 12N). A small piece of litmus paper added to the contents of the flask serves as the most convenient indicator. Cool the flask and its contents to about 30° to 40° C. and add concentrated sulphuric acid, drop by drop, until the solution is again distinctly acid (care should be taken that no drops of sodium hydroxide solution remain on the inside of the neck of the flask, which should be well washed down with water, or the flask may be stoppered and shaken). Now add from a burette a saturated solution of sodium hydrogen carbonate, until the solution becomes distinctly alkaline and an excess of 5 to 10 cubic centimeters of the reagent is present.

To this solution, add 2 cubic centimeters of a 1 per cent solution of starch, and titrate the arsenious acid present with N/20 iodine volumetric solution. Toward the end of the reaction, the solution usually develops a reddish-violet tint, which fades on standing. The end-point, however, is reached when the solution acquires the characteristic deep blue color given by free iodine in the presence of starch. From the amount of iodine consumed, the percentage of arsenic present is easily calculated. One cubic centimeter of N/20 iodine volumetric solution is equivalent to 0.001875 gram of arsenic.

Gravimetric method: Weigh out accurately about 0.2 gram of the product and transfer it to a Kjeldahl flask of 300 cubic centimeters capacity. Add 1.5 grams of a mixture of equal parts of sodium nitrate and potassium nitrate, 200 cubic centimeters of distilled water and 5 cubic centimeters of concentrated sulphuric acid. Heat the mixture slowly under a hood to allow the escape of the nitric acid fumes. Add a small quantity of concentrated or fuming nitric

acid from time to time, until oxidation is completed, which is generally indicated by the disappearance of the yellow color.[1] Continue the digestion until the volume of the liquid has been reduced to about 15 cubic centimeters,[2] cool, add 100 cubic centimeters of distilled water and again concentrate to about 15 cubic centimeters, in order to remove the last trace of nitric acid. If the product has been completely oxidized and all traces of nitric acid have been removed, the liquid will be water clear at this point. After cooling, cautiously neutralize the liquid with strong ammonia water and transfer it to a 300 cubic centimeter beaker, using a small quantity of distilled water for rinsing the flask.

To the solution, which will now contain all of the arsenic in the form of arsenate, add 10 to 20 cubic centimeters of 2N ammonium chloride solution for every 50 cubic centimeters of the liquid, then 20 cubic centimeters of magnesia mixture, drop by drop, with constant stirring. Finally add an amount of strong ammonia water, equal to one-third the volume of the liquid, and 2 cubic centimeters of alcohol. After allowing the mixture to stand for 12 hours, collect the precipitate, with the aid of a suction pump, in a Gooch crucible, which has been prepared as follows:

Cover the bottom of the crucible with a thin layer of asbestos, which has previously been washed with ammonia water (2.5 per cent), and dry in an oven at 110° C. Remove the crucible from the oven and place it in a larger porcelain crucible, fitted with an asbestos ring so that the sides and bottom of the two will not touch, put on the cover and heat slowly over an open flame until there is a light red glow on the outer crucible (Treadwell-Hall, 1905). Remove the Gooch crucible, cool in a desiccator and weigh.

After the precipitate has been collected, dry the crucible as described above, but add a crystal of ammonium nitrate before heating over the open flame. Finally cool the crucible and weigh. The weight of the precipitate multiplied by 0.48275 represents the amount of arsenic present in the sample taken for analysis.

Lehmann's method: Weigh out accurately about 0.2 gram of the substance and transfer to a 200 cubic centimeter Erlenmeyer flask.[3] Add 1 gram of finely powdered potassium permanganate and 5 cubic centimeters of dilute sulphuric acid and allow the mixture to stand for about 10 minutes. Rotate the flask frequently during this time to insure the complete mixing of the materials. Now add 10 cubic centimeters of concentrated sulphuric acid, in portions of about 2 cubic centimeters, rotating the flask after each addition.

[1] Sometimes the liquid may still have a pale yellow tint.

[2] Concentration should be effected in such a manner that the formation of sulphuric acid fumes in large quantities will be avoided.

[3] An Erlenmeyer flask, fitted with a glass stopper, is most suitable for this purpose.

When the reaction has ceased, add a quantity (about 5 to 7 cubic centimeters) of hydrogen dioxide solution sufficient to dissolve all of the brown precipitate. Toward the end, the hydrogen dioxide solution should be added, drop by drop, to avoid any great excess. Dilute the liquid with 25 cubic centimeters of distilled water and boil over wire gauze for about 10 minutes, or until the excess of hydrogen dioxide has been completely removed.[1]

After dilution with 50 cubic centimeters more of distilled water, cool the solution and add 2.5 grams of potassium iodide. Stopper the flask tightly and allow it to stand in a cool place for 1 hour. Finally titrate the liberated iodine with N/10 sodium thiosulphate volumetric solution without the use of starch test solution as an indicator.[2] One cubic centimeter of N/10 sodium thiosulphate solution is equivalent to 0.003748 gram of arsenic.

TABLE 1.—*Arsenic content of commercial samples of arsphenamine.*

Manufacturer.	Name of product.	Lot number.	Per cent of arsenic.			
			Direct titration with N/20 iodine V. S.	Ewins's method.	Gravimetric method.	Lehmann's method.
Dermatological Research Laboratories, Philadelphia, Pa.	Arsenobenzol	630	.....	30.06	.....	.....
Do	do	652	.....	29.61	.....	.....
Do	do	721	.....	29.11	.....	.....
Do	do	740	29.92	30.33	31.58	31.32
Do	do	750	29.24	.....	31.16	31.34
Do	do	755	29.34	29.26	31.13	30.94
Do	do	757	29.43	29.90	.....	.....
Do	do	767	29.53	29.20	.....	.....
Do	do	788	29.38	29.27	.....	.....
Do	do	791	29.19	29.26	.....	.....
Do	do	799	29.29	29.69	31.52	31.40
Do	do	809	29.95	29.59	30.87	30.46
Do	do	826	.....	29.20	.....	.....
Do	do	841	29.28	29.19	31.54	31.38
Do	do	845	29.07	29.30	.....	.....
Do	do	862	29.52	29.91	.....	.....
Do	do	873	29.53	29.71	31.38	31.22
Do	do	875	.....	29.74	.....	.....
Do	do	886	29.53	30.22	31.46	31.18
Do	do	890	29.23	.....	31.35	31.22
Do	do	900	29.42	29.70	31.07	30.94
Do	do	914	.....	28.71	.....	.....
Do	do	928	29.56	30.06	31.17	31.03
Do	do	952	29.62	30.33	31.07	31.03
Do	do	954	.....	31.24	.....	.....
Do	do	966	.....	29.45	.....	.....
Do	do	973	.....	30.51	.....	.....
Do	do	980	.....	.....	.....	30.46 30.52
Do	do	1008	.....	.....	.....	30.56 30.63
Do	do	1013	.....	.....	.....	30.44 30.72
Do	do	1017	.....	.....	.....	31.16 31.00

[1] Experience has shown that it is practically impossible to remove all of the hydrogen dioxide by boiling, unless the solution be evaporated to a very small volume, when it is very liable to become colored brown, due to the further action of the hot concentrated acid. In the analyses made by the authors the last trace of hydrogen dioxide was removed by the addition of a drop or two of permanganate solution (1 per cent) and the resulting pink color removed by the addition of oxalic acid solution in very slight excess.

[2] A blank test should be carried out under exactly the same conditions and the proper corrections made. The blank tests usually consume from 0.1 to 0.3 cubic centimeter of the iodine solution.

TABLE 1.—*Arsenic content of commercial samples of arsphenamine*—Continued.

Manufacturer.	Name of product.	Lot number.	Per cent of arsenic.			
			Direct titration with N/20 iodine V. S.	Ewins's method.	Gravimetric method.	Lehmann's method.
Dermatological Research Laboratories, Philadelphia, Pa.	Arsenobenzol	1020				31.27 31.08
Do	do	1048				30.38 30.40
Do	do	1062				30.41 30.61
Do	do	1072				30.15 30.26
Do	do	1077				30.53
Do	do	1095				30.57
Do	do	1105				30.63
Do	do	1125				30.49
Do	do	1135				30.82 30.73
Do	do	1142				30.53
Farbwerke-Hoechst Co., at H. A. Metz Laboratories (Inc.), New York.	Salvarsan	BDB	29.29	31.10		
Do	do	BFB	30.69	31.30		
Do	do	BJB	29.02	31.38		
Do	do	BLB	29.40			
Do	do	BMB	29.88	29.98		
Do	do	BUB	29.34	30.88		
Do	do	BVB	29.60	31.44		
Do	do	BXB	29.43	30.35		
Do	do	DBB	28.87	29.69	31.16	31.22
Do	do	DFB	29.97	31.30	31.47	31.32
Do	do	DHB	30.05	30.76 31.22		
Do	do	DHB	30.24	30.76		
Do	do	DJB	29.60	31.05		
Do	do	DLB	30.63	31.95		
Do	do	DMB	29.07 29.62	30.72 29.57		31.50
Do	do	DUB	29.16	30.31		
Do	do	FBB	29.52	31.00 31.54	31.85	31.50
Do	do	HBB	30.74	31.64	31.65	31.28
Do	do	JBB		30.43		
Do	do	LBB			31.24	31.20
Do	do	MBB				32.24 32.24 32.06 32.24 32.06 32.19
Do	do	UBB				31.29 31.27
Do	do	XBB				30.92 30.85
Do	do	I75			31.15	30.63 30.72
Do	do	I76			31.15	31.52 31.58 31.58
Do	do	I77			31.80	31.64 31.58 30.82
Do	do	I78				30.82 30.91 30.94
Do	do	I79			31.56	31.36 31.58
Do	do	I81				32.15
Do	do	I82				31.97
Les Etablissements Poulenc Frères, Paris.	Arsenobenzol "Billon."	D632		29.93		30.74
The Diarsenol Co. (Ltd.), Toronto, Canada.	Diarsenol	BS7520	28.89	29.92		
Do	do	BS7521	29.05			30.18
Do	do	1255				30.73
Fankyo & Co., Tokyo	Arsaminol	68	29.86	31.26		31.94
Do	do	54	29.89	31.20		32.26
Arsemin Co., Tokyo	Neo Neo Arsemin (Salvarsan sod.)	DEIA		21.04		
Do	do	DEIS		20.73	20.47	20.50

TABLE 2.—*Arsenic content of commercial samples of neo-arsphenamine.*

Manufacturer.	Name of product.	Lot number.	Per cent of arsenic.		
			Ewins's method.	Gravimetric method.	Lehmann's method.
Farbwerke vorm. Meister Lucius & Bruning, Hoechst a. M.	Neosalvarsan	HV	18.38	19.82	20.12
Les Etablissements Poulenc Frères, Paris.	Novarsenobenzol "Billon"	B1539	17.80	20.34	19.93
Do	do	B2126	18.98		20.21
Do	do	B2137	18.06	19.96	19.74
Do	do	8750	18.19	20.35	19.93
Do	do	9651	18.24	20.05	20.12
Anglo-French Drug Co. (Ltd.), London.	Ampsalvs		18.28		19.81 19.65
Kokusan-Seiyakusho, Tokyo	Neotanvarsan	19	18.26		
Do	do	20	18.15	18.27 18.34	18.40
Do	do	21	18.10	18.19	18.30
Banyu Co., Tokyo	Neochramisol	CHA	18.82		[a] 17.93
Do	do	CHA	18.47		18.41
Sankyo & Co., Tokyo	Neoarsaminol	N139	16.56	16.96	17.04
Do	do	N153	16.81		16.70
Do	do	N183			17.21 17.44 17.27
Do	do	N185	16.80	16.96	16.89
Synthetic Drug Co., Toronto	Neodiarsenol	180			16.69
Do	do	181			15.50
Do	do	182			16.68
Do	do	183			17.55
Do	do	189		15.79 16.05	[b] 15.33
Do	do	262			16.35
Do	do	264			15.37 15.29
Do	do	267			15.46 15.30

[a] The tube had been opened for a considerable length of time previous to analysis and the product was oxidized to a considerable extent.
[b] The sample was not uniform.

A survey of the preceding tables shows that the results obtained by the Lehmann and the gravimetric methods are nearly identical, while those obtained by direct titration with iodine volumetric solution are relatively low in all cases. With the Ewins method, the results are occasionally of the same magnitude as those obtained by the gravimetric determination, but, as a rule, they are also relatively low.

With respect to the titration method, Gaebel (1911c) states that the reaction between arsphenamine and iodine is a reversible one, viz:

$$C_{12}H_{12}O_2N_2As_2.2HCl.2H_2O + 8I + 4H_2O \rightleftarrows 2C_6H_8O_4NAs.HCl + 8HI.$$

As a consequence a state of equilibrium is reached before all of the arsphenamine has been oxidized and the amount of iodine solution consumed is less than that required by theory. This investigator states further that the reagents (sodium bicarbonate, sodium acetate, borax, etc.) usually employed for overcoming this difficulty in iodometric titrations of arsenious compounds are of no value in this case, a condition which has also been observed by the authors. This method appears, therefore, to be of little value.

The low percentages obtained by the Ewins method are apparently the result of a loss of arsenic through volatilization. It was thought that this loss might be avoided by slowing the rate of digestion. A number of samples were, therefore, digested for some time in the cold and then slowly over a low flame. Samples from the same tubes were also digested rapidly in order to obtain data for comparison. The results obtained follow;

TABLE 3.—*Effect of rate of digestion on the results obtained by the Ewins method.*

Manufacturer.	Name of product.	Lot number.	Per cent of arsenic, Ewins's method.	
			Slow digestion.	Rapid digestion.
Dermatological Research Laboratories, Philadelphia	Arsenobenzol.	740	30.33	28.64
Do	do	750	.....	28.75
Do	do	799	29.69	28.60
Do	do	873	29.71	28.69
Do	do	886	30.22	28.52
Do	do	890	.....	28.65
Do	do	952	30.33	28.62

The above data indicate that the rate at which digestion is allowed to proceed is a factor which influences the final result to a very considerable extent. But they also show that the results are low even when digestion is carried out very slowly. It appears, therefore, that this method in its present form is objectionable. It is possible that greater accuracy might be attained by condensing the fumes which escape during digestion, reuniting the distillate with the contents of the Kjeldahl flask previous to neutralization, and finally titrating the mixture. Work along this line is, however, necessary before a positive statement can be made.

The method of Lehmann, with the slight modifications recommended in the footnotes, is accurate and reliable. It is simple, requires but small quantities of inexpensive reagents, and can be completed in about one and one-half hours. It, therefore, appears to be superior to any of the other methods mentioned for the routine analysis of these products.

Bibliography.

ABELIN, J.
1911. Ueber eine neue Methode, das Salvarsan nachzuweisen. Münch. med. Wchnschr., v. 58, p. 1002–1003.

AMERICAN MEDICAL ASSOCIATION (Council on Pharmacy and Chemistry).
1917. New and Nonofficial Remedies, Chicago, Am. M. Assoc.

BOHRISCH, P., and KÜRSCHNER, F.
1911. Zur quantitätiven Bestimmung des Arsens organischen Substanzen, mit besonderer Berücksichtigung organischer Arsenverbindungen (Salvarsan usw.). Pharm. Zentralh., v. 52, p. 1365–1369.

DENIGÈS and LABAT.
1911. Réactions et dosage de l'arsénobenzol ou 606. Répert. de Pharm., v. 23, p. 251–253.
DENIGÈS and LABAT.
1913. Réactions et dosage du néosalvarsan. Répert. de Pharm., v. 25, p. 9–10.
EHRLICH, P., and BERTHEIM, A.
1912. Ueber das salzsaure 3,3′-Diamino-4,4′-dioxyarsenobenzol und seine nächsten Verwandten. Ber. deutsch. chem. Gesellsch., v. 45, p. 756–766.
EHRLICH, P., and PAULY, H.
1904. Ueber die Konstitution des Histidins. Ztschr. physiol. Chem., v. 42, p. 508.
EWINS, ARTHUR J.
1916. The Estimation of Arsenic in Organic Compounds. J. Chem. Soc. (Trans.), v. 109, pt. 2, p. 1355–1358.
FOLIN, OTTO, and DENIS, W.
1912. On Phosphotungstic-Phosphomolybdic Compounds as Color Reagents. J. Biol. Chem., v. 12, p. 240.
GAEBEL, G. OTTO.
1911a. Die quantitative Zusammensetzung des Salvarsan. Apoth.-Ztg., v. 26, p. 215–216.
GAEBEL, G. OTTO.
1911b. Das Salvarsan beim gerichtlichen Arsennachweis. Arch. Pharm., v. 249, p. 49–56.
GAEBEL, G. OTTO.
1911c. Titration von Salvarsan mit Jodlösung. Arch. Pharm., v. 249, p. 241–247.
GIUSEPPE, BRESSANIN.
1911. Methode zum Nachweis und zur Bestimmung von Arsen in organischen Verbindungen. Chem. Centralbl., v. 82, p. 1965, from Boll. Chim. Farm., v. 50, p. 727–730.
JANNASCH, P., and SEIDEL, J.
1910. Ueber die quantitative Verflüchtigung des Arsens aus Lösung unter Reduktion des Arsenchlorids zu Arsenchlorür durch Hydrazinsalze. Ber. deutsch. chem. Gesellsch., v. 43, p, 1218–1223.
LA COSTE, W. and MICHAELIS, A.
1880. Ueber aromatische Arsenverbindungen. Ann. Chem., v. 201, p. 224.
LEHMANN, F.
1912. Ueber die Bestimmung des Arsens in Salvarsan und Neosalvarsan. Apoth.-Ztg., v. 27, p. 545–546.
LITTLE, HARRY F. V., CAHEN, EDWARD, and MORGAN, GILBERT T.
1909. The Estimation of Arsenic in Organic Compounds. J. Chem. Soc. (Trans.), v. 95, p. 1477–1482.
MARTIN, E. H.
1916. Alarming Symptoms Caused by Diarsenol. J. Am. M. Assoc., v. 66, p. 1155.
MOELLER, JOSEPH and THOMS, HERMANN.
1914. Real-Enzyklopädie der Gesamten Pharmazie, v. 14, p. 35.
NORTON, F. A. and KOCH, A. E.
1905. A Method for the Detection and Determination of Arsenic and Antimony in the Presence of Organic Matter. J. Am. Chem. Soc., v. 27, p. 1247–1251.

ORMSBY, OLIVER S. and MITCHELL, JAMES H.
1916. Toxicity of the Present Supply of Salvarsan and Neosalvarsan. J. Am. M. Assoc., v. 67, p. 1756.

FRINGSHEIM, HANS H.
1904. The Analysis of Organic Substances with the Help of Sodium Peroxide. Am. Chem. J., v. 31, p. 386–395.

PUCKNER, W. A. and HILPERT, W. S.
1910. The Chemical Properties of Salvarsan ("606"). J. Am. M. Assoc., v. 55, p. 2314.

RUPP, E. and LEHMANN, F.
1911. Ueber eine vereinfachte Bestimmung des Arsens in Atoxyl und Arsacetin. Apoth.-Ztg., v. 26, p. 203–204.

SCHAMBERG, JAY F., KOLMER, JOHN A., and RAIZISS, GEORGE W.
1917. Experimental and Clinical Studies of the Toxicity of Dioxydiamino-Arsenobenzol Dihydrochloride. Reprint from J. Cutaneous Dis., May-June, p. 1–52.

ST. WARUNIS, THEODOR.
1912. Bestimmung des Arsens in organischen Verbindungen. Chem. Ztg., v. 36, p. 1205–1206.

SCHNEIDER and FYFE.
Quoted by Schmidt, 1906, Pharmazeutische Chemie. Friedr. Vieweg. & Sohn, Braunschweig, v. 1, p. 379.

TREADWELL-HALL.
1905. Analytical Chemistry. John Wiley & Sons, New York, v. 2, p. 25.

WILLCOX, WILLIAM H. and WEBSTER, JOHN.
1916. The Toxicology of Salvarsan, Dioxy-diamino-arsenobenzol. Brit. M. J., v. 1, p. 474.